Environmental & Occupational & Injury Prevention Coordinating Center

Top priorities:

Newborn Screening Program

Newborn screening, a major public health responsibility, is the largest genetic testing effort in the nation. Effective screening of newborns begins with the accuracy and reliability of the screening test, followed by diagnostic studies and treatment. For more than 25 years, the Division of Laboratory Sciences (DLS) at CDC's National Center for Environmental Health with its cosponsor, the Association of Public Health Laboratories, has conducted research on materials development and assisted laboratories with quality assurance (QA) for these screening tests. CDC's Newborn Screening Quality Assurance Program (NSQAP) is designed to help screening laboratories achieve excellent technical proficiency and maintain confidence in their performance while processing large volumes of specimens daily.

The NSQAP provides QA services for congenital hypothyroidism, phenylketonuria, galactosemia, congenital adrenal hyperplasia, maple syrup urine disease, homocystinuria, biotinidase deficiency, galactose-1-uridyltransferase (GALT) deficiency, cystic fibrosis, and hemoglobinopathies (including sickle cell disease). QA services are also provided for fatty acid oxidation and organic acid disorders detected by tandem mass spectrometry (MS/MS). The program prepares and distributes more than 500,000 dried blood spots (DBS) to national laboratories every year. These materials must simulate as closely as possible the actual specimens for the assay systems and are certified for homogeneity, accuracy, stability, and suitability for all assays from different commercial sources. NSQAP is the sole source of these comprehensive QA services worldwide.

National Health and Nutrition Examination Survey (NHANES) DNA Bank

The National Health and Nutrition Examination Survey (NHANES) is a program of periodic examination surveys conducted by CDC's National Center for Health Statistics (NCHS) that provides nationally representative information on the health and nutritional status of the U. S. population. CDC's NHANES DNA bank was conceived by scientists in NCEH in 1988 in response to the newly established Human Genome Project to map and sequence the human genome and to the emergence of new technologies such as the polymerase chain reaction (PCR) and automated DNA sequencing.

The intent in establishing CDC's NHANES DNA bank was to create a representative genetic sample of the U.S. population for intramural and extramural research. Immortalized cell lines were established because current technology would require large amounts of DNA, and these cell lines provided a virtually unlimited source of DNA. The DNA Bank consists of 8,000 immortalized B-lymphocyte cell lines. Approximately 14,000 white cell samples that were not immortalized were stored in liquid nitrogen and

could be used as a back up for immortalization or as a direct source of DNA. In addition, approximately 12,000 blood clots have also been frozen that also could be used as a source of DNA.

Prevalence of gene variants that code for enzymes involved in nicotine and carcinogen metabolism

Cotinine, a metabolite of nicotine, is a reliable measure of exposure to cigarette smoke. People convert the harmful chemicals in cigarette smoke into potentially harmful substances differently, depending on their genetic make-up.

To address the question of whether or not genetically-based differences in nicotine metabolism account for differences in cotinine levels among individuals or groups, the Molecular Biology Branch of NCEH's Division of Laboratory Sciences will genotype approximately 7,300 samples from the NHANES III DNA Bank for 14 polymorphic genes which code for enzymes that are associated with the metabolism of nicotine and other toxicants in cigarette smoke or are associated with smoking characteristics. Each of the proposed gene variants have been implicated in smoke-related and other cancers or in smoking habits among people.

This study will compare the body burden of cotinine and other parent chemicals from cigarette smoke among people and among racial or ethnic groups by genotype. In addition, the number of cigarettes smoked per day and smoking history will also be examined.

This information will help explain the potential benefits and risks associated with having one or more of the gene variants as these variants relate to the effects of smoking on health, smoking habits (including the ability of people to successfully stop smoking), and to targeted screening and intervention programs. The results of this study will help explain the documented differences in cotinine levels in the U.S. population and specific ethnic groups in that population

Churchill County Leukemia Cluster Investigation

As part of its response to an investigation of a cluster of cases of acute lymphocytic leukemia among children in Churchill County, Nevada, the Nevada State Health Division (NSHD) requested technical assistance from the Centers for Disease Control and Prevention (CDC). The purpose of the subsequent collaborative investigation was to conduct a cross-sectional exposure assessment to identify contaminants unique to the Churchill County community.

One part of that investigation involves using DNA obtained from study participants to look for differences among genes that may affect susceptibility to leukemia. For example, a gene that directs the production of the protein called MTHFR has at least two

different forms in the United States population. MTHFR is important in the body's production of folic acid, a vitamin needed to prevent birth defects and that may also be

important in preventing cancer and heart disease. One form of MTHFR, although it does not cause birth defects, can increase the risk for birth defects, such as spina bifida, in the presence of environmental factors and may be related to the risk for cancer.

Some scientific evidence shows that having one of these forms of MTHFR may protect people against developing acute lymphoblastic leukemia. MTHFR is only one of about 15 genes that CDC plans to study for this type of variation among people that, in the presence of certain environmental exposures, such as exposure to pesticides, may affect the risk for leukemia. This work is being done to determine whether or not the Churchill County children with leukemia or their family members are more likely to have one form of the gene, whereas children and their families in the control group are more likely to have another form of the gene.

The National Birth Defects Prevention Study Centralized Laboratory

The National Birth Defects Prevention Study (NBDPS) is one of the largest case-control studies of birth defects ever conducted in the United States. The goal of this ongoing multi-center study is to identify environmental and genetic risk factors for birth defects. Information on environmental risk factors is collected through a maternal interview, and DNA is collected from the infant and both parents for evaluation of genetic risk factors.

Cheek cell (buccal) specimens are obtained because of their low cost and ease of use for sampling infants without the direct involvement of health-care workers. However, unlike whole blood, buccal specimens yield lower amounts of DNA that is often of poorer quality. Because of these limitations, the best DNA banking and quality-control practices must be employed in establishing buccal DNA collections.

Initially, each Center was responsible for DNA isolation and quality control. However, the laboratory proficiency testing program demonstrated that considerable variation existed in the technical ability of the laboratory staff of the various centers participating in the study, and there was also variation in laboratory results. Moreover, standardization of methods for isolating DNA and for ensuring quality control was lacking.

To ensure the integrity of the biological data, the decision was made to consolidate the biologic processing to a single laboratory dedicated to this activity. In 2003, the NBDPS Centralized Laboratory was established in the Molecular Biology Branch of the Division of Laboratory Sciences, NCEH. Key features of this laboratory include a staff with extensive expertise in DNA banking and performance of laboratory operations that satisfy CLIA regulations, an extensive and integrated data-management system, and an array of state-of-the-art instrumentation. This activity is further enhanced by an active research program in emerging laboratory technologies.

Genetics of Kidneys in Diabetes (GoKinD) Study

Diabetes Mellitus is the leading cause of treated end-stage renal disease (ESRD), accounting for 43% of new cases. About 40% of those with type 1 diabetes (T1D) develop severe kidney disease and ESRD by the age of 50. The health-care expenditures for the renal complications of diabetes were $1.9 billion for 2002. This burden of illness and cost reinforces the need for strategies to prevent the development of renal disease in those who are at risk.

The role of genetic risk factors in the etiology of renal disease is supported by 1) evidence for family clustering of renal disease in a wide variety of ethnic groups with various predisposing conditions; 2) the fact that hyperglycemia alone is not sufficient to cause renal disease; and 3) family histories that indicate that hypertension, cardiovascular disease, and possibly insulin resistance predispose to nephropathy. In various populations, renal disease clusters in families independent of predisposing conditions, such as diabetes, lupus erythmatosis, or HIV infection, indicating that genetic risk factors for diabetic renal disease may differ from those for diabetes itself.

The GoKinD Study is a collaboration of the Juvenile Diabetes Research Foundation (JDRF); its Coordinating Center, consisting of the Joslin Diabetes Center and George Washington University Biostatistics Center; and CDC. GoKinD is designed as a collection of samples from clearly delineated case participants with type 1 diabetes who also have renal disease and from control participants with T1D who do not have renal disease but who excrete very small amounts of albumin. The intent of the collaboration is to facilitate the study of genetic risk factors for diabetic renal disease. Half of the participants were recruited by the Joslin Diabetes Center and half by clinical centers associated with the George Washington University Biostatistics Center. The clinical centers and the University of Minnesota provided extensive clinical data that characterized these participants, and the Molecular Biology Branch in the Division of Laboratory Sciences at NCEH provided genetic data on major T1D risk factors. CDC is also conducting additional research on the collection. Recruitment is complete, and sample distribution to other approved researchers will begin in 2005.

Case-control study of environmental exposures and genetic susceptibility among individuals with multiple sclerosis living in three geographic areas

Although multiple sclerosis (MS) is the most common neurologic disease disabling young adults in the United States, the cause of this disease is unknown. Evidence indicates that it is a complex disease with a multifactorial etiology determined by both environmental factors and genetic susceptibility. However, previous research examining potential causes of MS has focused on either the role of environmental exposures or the role of genetic factors but not on a combination of the two. This study examines the joint role of selected environmental exposures and candidate genes as potential risk factors for MS.

In November 2004, study investigators began enrolling 1500 individuals (500 case and 1000 control subjects). Case subjects will include individuals who have been diagnosed with MS and were identified through the prevalence studies that were recently completed in Ohio, Missouri, and Texas by local and state health departments under a cooperative agreement with ATSDR. Control subjects will be selected from patients who attended the same neurologist's office from which the cases arose. The diagnoses for control selection will be limited to those for which there is no prior indication of a relation with the exposures under study.

Each participant in this study will complete a questionnaire and provide a blood sample. Information collected in the questionnaire will include exposure to potential environmental factors thought to be associated with MS such as exposure to heavy metals, solvents, and other toxic chemicals, as well as a complete residential history, school history (location of), occupational history, and hobbies/lifestyle exposures. Investigators also will collect information on family medical, history, reproductive, and smoking histories. Case subjects will complete one extra section about the course of their disease. A blood sample will be obtained from all participants that will be genotyped for specific genes thought to be associated with MS, including human leukocyte antigen, immunoglobulin heavy chain, T-cell receptor, tumor necrosis factor, and myelin basic protein. A metabolomic analysis will also be conducted on a sub-set of blood samples.

Major accomplishments, 2004

Newborn Screening Program

The QA services provided by CDC's Newborn Screening Program primarily support newborn screening tests performed by state laboratories; however, the program also accepts other laboratories and international participants. All laboratories in the United States that screen newborns participate voluntarily in NSQAP. NSQAP participants can use the program's Web site (www2.cdc.gov/nceh/newbornScreening) to report their data and receive performance evaluations. Over the last few years, NSQAP has grown substantially, both in the number of participants and in the scope of global participation. In 2004, 400 laboratories in 60 countries (at least one laboratory per country) were active program participants. NSQAP currently provides QA services for 35 disorders.

In January 2003, the program began distributing five-specimen panels for Type 1 Diabetes composed of dried blood spots from a validated specimen library of sequenced patient samples. Four research laboratories that do population-based testing participate in the pilot PT to assure comparability of data from the different research sites. The program has now added a DNA confirmatory testing component to the testing materials for cystic fibrosis, producing our second phenotype/genotype combination DBS. Additionally, NSQAP is investigating the development of specimens for detecting toxoplasmosis antibodies in DBS using serum from infected individuals and is conducting pilot testing to determine the feasibility of using these specimens to establish a proficiency testing program.

National Health and Nutrition Examination Survey (NHANES) DNA Bank

In 1999, DNA was made available to the research community. The Division of Laboratory Sciences, in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP), conducted a pilot study to assess the prevalence and penetrance of mutations related to hereditary hemochromatosis. This disease is mainly associated with mutations in a gene called *HFE*, which helps regulate the amount of iron absorbed from food. The defect can result in iron overload that can lead to organ damage. If a diagnosis is made early in the course of disease, organ failure can be prevented by inducing a mild anemia through the periodic removal of blood. Hereditary hemochromatosis is one of the most common genetic disorders in the United States. It most often affects Caucasians of Northern European descent, although other ethnic groups are also affected. The pilot study provided the first estimate of the prevalence of these mutations in a representative sample of the United States population. This information is for making policy decisions about screening health populations for hereditary hemochromatosis.

DLS and NCCDPHP collaborators published the first studies using NHANES III DNA bank samples, the aforementioned work related to prevalence of mutations that cause hereditary hemochromatosis, and one on the association of the HFE mutations with levels of iron in the blood. The results of this study show that although the presence of the mutations is associated with elevated iron levels, a substantial proportion of people with the mutations do not have high levels of iron. The study reaffirms the need for additional information about the risk of iron overload and chronic disease associated with HFE mutations, as well as other genetic and environmental factors that modify this risk in order to make informed decisions regarding genetic screening for hemochromatosis.

Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA, Grummer-Strawn LM, Sampson EJ, Khoury MJ, Gallagher ML. The prevalence of C282Y and H63D mutations to the hemochromatosis (HFE) gene in the United States. *JAMA*. 2001;285(17):2216-2222.

Cogswell ME, Gallgher ML, Steinberg KK, Caudell SP, Looker A, Bowman B, Gunter E, Franks AL, Khoury MJ, Grummer-Strawn LM. The HFE genotype and transferrin saturation in the United States. Genetics in Medicine. 2003;5(4) 5(4):304-10.

NHANES 1999-2003 Survey:
The current NHANES survey began in 1999. We have obtained DNA from whole blood specimens obtained from 8,000 subjects aged 20 yrs and older. These additional 8,000 DNA samples will significantly expand the existing NHANES DNA bank and will be an important resource for intramural (CDC) and extramural genetic research programs.

Prevalence of gene variants that code for enzymes involved in nicotine and carcinogen metabolism

Major accomplishments for 2004 include the development of robust, high throughput assays which will be used to determine the genotypes of the selected variants present in the 14 polymorphic genes. Each assay is subjected to a rigorous validation process to verify the accuracy of the assay. Extensive quality control procedures were developed. Both of these components of the study will ensure that the highest quality of data is produced.

Churchill County Leukemia Cluster Investigation

MTHFR and several other genes have been tested for the presence of known DNA polymorphisms (gene variants) and analysis of the results is in progress. Elevated levels of several metals were found in the Churchill County population. Although these metals have not been associated with an increased risk of leukemia, several enzymes that may play a role in formation of blood cells are influenced by metals such as tungsten. The genes that produce three of these enzymes were studied in depth in order to discover all of DNA polymorphisms present in these genes. A number of new gene variants were identified and analysis of the results is in progress.

The National Birth Defects Prevention Study Centralized Laboratory

Over 5,000 specimens were received last year. Since its inception, the NBDPS Centralized Laboratory has received at least 6,000 buccal specimens, and 95.1 % of DNA samples have successfully passed the quality-control process. This high success rate will enhance the utility of this resource in investigations of genetic risk factors for birth defects.

Genetics of Kidneys in Diabetes Study (GoKinD)

GoKinD has recruited over 2,700 participants including 243 case subjects with two parents (trios), 283 control-subject trios, 559 case-subject singletons, and 565 control-subject singletons. In all, this collection includes 802 individuals with both T1D and renal disease and 848 individuals with T1D and no renal disease. It will also include 1650 individuals with T1D and 526 T1D trios. CDC authors have published four papers and made seven presentations at major meetings on GoKinD. A draft of the baseline paper for the study is complete and ready for clearance and submission, and analysis of the HLA data for DRB1, DQA1, and DQB1 and the insulin gene data is in progress.

CDC has completed one half of a pilot study of HLA matched case and control subjects using the Affymetrix 10,000 single nucleotide polymorphism micoarray to identify regions of the genome associated with renal disease. In addition, CDC is conducting a study of type 1 diabetes and renal disease candidate genes and is extending the HLA genotyping to DPB1 and HLA Class I B.

Future directions:
In addition to continued work on the programs and studies that are current top priorities in the Coordinating Center, the following future directions have been identified to meet priority needs.

Asthma

Asthma is a major public health problem that affected 20.3 million Americans in 2001. The estimated cost of treatment of those younger than 18 years of age is $3.2 billion per year. Asthma disproportionately affects low-income populations, minorities, and children living in inner cities. Currently there are no preventative measures or cure for asthma. Despite intensive investigation, the factors that confer susceptibility are not well understood, but it is clear that both environmental and genetic factors each account for about 50% of the risk. A better understanding of the interaction of environmental risk factors with genetic risk factors is crucial to developing prevention strategies and interventions to decrease the impact of this disease.

A case-control study of sufficient size involving patients drawn from representative populations with carefully described phenotypes of asthma, supporting clinical data, and well-documented environmental exposures would offer a valuable means of advancing this field. Such a study would allow genetic and environmental risk factors to be studied objectively to determine the relative size of their effects and how they interact.

Clusters

CDC has long recognized the need to respond to public concern about cancer clusters, while recognizing the low probability of finding causes of cancer in community clusters. Over a decade ago, CDC organized The National Conference on Clustering of Health Events and published the proceedings of that meeting (Neutra, 1990). CDC also released "Guidelines for Investigating Clusters of Health Events" (CDC, 1990), and variations of these guidelines are still used in cluster investigations. These deliberations also identified deficiencies including lack of documentation of exposures.

Today, CDC has added state-of-the-science laboratory methods for comprehensive investigations of cancer clusters. These methods have enabled CDC to document and assess environmental exposures by comparing them to exposures in the general population. CDC is also using molecular methods to study the effects of documented environmental exposures. For example, for one cluster of acute lymphoblastic leukemia in children, CDC documented excessive exposures to environmental chemicals and recommended steps to avoid those exposures. CDC then looked for genetic differences between case and control children that might make some more susceptible than others to the effects of these toxicants and possibly alter susceptibility to leukemia.

In the future, CDC will introduce molecular methods to study differences in susceptibility to environmental toxicants. Further, CDC will work to assure collection of important diagnostic information on molecular lesions in patient's bone marrow before the patients are treated with chemotherapy. These lesions, including translocations and mutations, have been reported to be associated with specific leukemias. This work will be done in an effort to look for common features that may add to evidence of a common exposure and perhaps someday help to identify "signature" molecular lesions for classes of exposure.

Newborn Screening

As states adopt new screening tests to detect additional diseases, DLS must maintain QA services for these new technologies. DLS plans to establish a counterpart of NSQAP, which will be called the Newborn Screening Translational Research Laboratory 1) to identify promising tests currently used in research settings that could be applied to public health newborn screening; 2) to develop laboratory methods for detecting in newborns medical conditions and risk factors that ultimately could benefit children's' health; 3) to evaluate new technologies, particularly high-throughput nanoscale methods, that could be applied to newborn screening; 4) to conduct pilot programs in collaboration with state newborn screening programs to assess and refine the practical application of new laboratory tests; 5) to develop QA/QC tools for new tests before they are implemented on a population basis; and 6) to assist epidemiologists in using tests on newborn DBS for population-based assessment of risk factors and disease prevalence.

Several ongoing activities will include developing QA/QC materials for disorders, such as genotyping DBS materials for cystic fibrosis; supporting research programs that use newborn screening to assemble cohorts for longitudinal studies on type 1 diabetes and related autoimmune disorders; developing a screening test for severe combined immunodeficiency (SCID); developing multiplexed ligand-binding assays to identify risk factors for neurobehavioral developmental disorders such as autism and Fragile X syndrome; and evaluating reverse microarray technology for high-throughput, low-cost genotyping.

Toxicogenomics: Use of gene expression arrays to document exposure to environmental toxicants with signature profiling of chemicals or to predict toxicity from these exposures.

Currently, scientists are doing basic research to identify phenotypic changes associated with the altered patterns of gene expression and to link those phenotypic changes to conventional measures of toxicity. Scientists must also define toxic doses and the latency period between expression of such signature genes and outcomes. This problem is especially difficult because of the multiple exposures that occur in communities. The work must first be done in animals with exposures to single chemicals.

NCEH is poised to translate the basic research findings of the National Center for Toxicology and others to define mechanisms of toxicity in order to predict the toxicity of environmental exposures. In cases where scientists identify signature molecular lesions or patterns of expression, CDC will carry out hypothesis-driven, epidemiologic research in exposed populations to confirm the predictive value of these expression profiles/lesions.